PNW-346 February 1980

EFFECT OF DATE OF CONE COLLECTION AND STRATIFICATION PERIOD ON GERMINATION AND GROWTH OF DOUGLAS-FIR SEEDS AND SEEDLINGS

by

Frank C. Sorensen, *Principal Plant Geneticist*

Abstract

Low-elevation seeds collected 6 and 2 weeks before assumed natural seed fall were stratified 1, 2, 4, 8, 16, 32, 64, and 128 days and germinated in the laboratory. Germinated seeds from all stratification periods were sown at the same time in the nursery bed. Germination and seedling measurements were taken over two growing seasons.

Early collection gave smaller seeds, reduced germination percentage, and yielded smaller seedlings. Reduced seedling size appeared to be related to reduced seed weight.

Germination rates of both early- and late-collected seeds increased with increased stratification, but the response of late-collected seeds was greater. When time to 50 percent of total germination and length of stratification both were expressed in logs, the response was linear over the range of stratification periods. Stratification beyond about 30 days was detrimental to total germination of early-collected seeds.

Total height of 1st-year seedlings was linearly related to the log of stratification period only for late-collected seeds. The effect was small and not present by the end of the 2d year. Stratification appeared to increase 1st-year seedling size through its effect on hypocotyl extension. Epicotyl elongation rates during the exponential phase of elongation and elongation period were not affected by stratification period.

KEYWORDS: Germination (seed), seeding date, stratification (seed), cone collection, seed weights, seedling growth, Douglas-fir, Pseudotsuga menziesii.

INTRODUCTION

Exposure to low, but above freezing, temperatures during a resting phase often increases the potential rate of development during subsequent growth periods. Chilling of seeds previously brought to the proper moisture content (stratification) may affect germination rate (Allen 1958, McLemore and Czabator 1961, Mergen 1963), and chilling of seedlings may affect rate of bud-burst (Wommack 1964, Nienstaedt 1967, Campbell and Sugano 1979).

The low-temperature effects may be limited to a relatively short time during plant development, for example to the elongation of embryos prior to germination or to elongation of buds prior to flushing; however, it may also extend to processess of longer duration. Rohmeder (1962) and Kriek (1976) present evidence that speed of germination, which can be influenced by chilling, also affects seedling size. Others have observed that absence of low-temperature treatment continues to influence stem elongation processes after germination (Flemion 1934, Pollock 1959) or after bud burst (Schwabe 1973, R. K. Campbell, USDA Forestry Sciences Laboratory, Corvallis, Oreg., personal communication 1979).

In most germination and growth tests, the speed of germination has been confounded with other factors such as genetic composition, seed size, or onset of germination so that causal relations between germination rate and other factors could not be positively identified. The purpose of the present study was to investigate the effects of germination rate on growth of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings using a known source of seeds and timing germination to occur together. Samples from a single lot of seeds were stratified for different periods ranging from 1 to 128 days, germinated seeds were sown at the same time, and seedling size was measured periodically. As the maturity of seed is known to affect germination rate, seed weight, and possibly chilling response (Maki 1940, Cram and Worden 1957, Allen 1958, Ching and Ching 1962, Rediske 1969), seeds of two maturities were also included. Specific objectives were:

1. Relate germination rate and seedling size through 2 years in the nursery to maturity of seed and eight stratification periods.
2. Determine the mathematical relationships between stratification period and both germination rate and seedling size.

The purpose of the first objective was to further evaluate the extent and ways in which seed maturity and stratification might confound genetic comparisons (Olson and Silen 1975). The second objective was important because of ongoing and planned work relating seed germination and seedling bud-break patterns of different geographic sources of Douglas-fir to climate at the seed origin.

MATERIALS AND METHODS

Cone Collection and Handling

Cones were collected in 1976 from five trees growing in a low-elevation stand in the western Oregon Cascades (latitude 44° 35'N, longitude 120° 42'W, 275-m elevation). An "early" collection of approximately 50 cones per tree was made August 11 when cone scales were green to slightly brown. A "late" collection of similar size was made September 9 when cones were brown to opening, depending upon the tree. Fresh weights of five-cone samples from each tree were determined. The cones were then dried 72 hours at 80°C in a circulating-air oven, reweighed, and moisture percentage determined on a dry weight basis.

The remaining cones were allowed to dry at 22°C (room temperature) until the cone scales flared. Seeds were extracted by striking the basal ends of individual cones with a stick. After extraction, seeds were de-winged and X-rayed. Filled seeds from each tree were weighed and placed in cold storage (-10°C) until required for stratification treatments and sowing the following spring.

Seed Stratification and Germination

Seeds soaked in water for 24 hours at 22°C were stratified at 3°-4°C. Eight stratification periods, 1 to 128 days, were used and were arranged such that each stratification period doubled the length of the previous period, e.g., 1, 2, 4, 8 days, etc. In this way, stratification periods were closer together when the most response to stratification could be expected, and also the periods were equally spaced on a logarithmic scale. After stratification, seeds were germinated on moistened filter paper in covered sandwich boxes in a controlled environment chamber at a constant 20°C with a 12-hour light period. Seeds from each tree were germinated in separate boxes placed at random in the growth chamber.

Sample size for each of the 16 pregermination treatments (2 seed maturities and 8 stratification periods) consisted of 156 filled seeds (35 seeds from 4 trees and 16 seeds from 1 tree which had low numbers of filled seeds).

Germination was observed for 4 months, after which ungerminated seeds were cut. Seeds with decayed gametophytes and embryos were considered nongerminable, and germination rate and percent for each dish was based on the number of germinated + firm filled seeds in the dish (Allen 1960).

Mean rate of germination for each dish was determined by plotting cumulative percentage against days^{-1} on probit paper (Campbell and Sorensen, in prep.). A straight line was visually fitted to the points and mean germination rate, R, read as days^{-1} at which observed germination equaled 50 percent of total germination. Germination rates for each stratification period and seed maturity were based on the average rate of all five families. Families were not replicated within treatments, and no test was made of family differences.

Log Transformation of Germination Rate

Germination is the culmination of many processes and cannot be attributed to one event or treatment. Nevertheless, as the basis for the log transformation, I assumed that a change in the length of stratification was accompanied by some proportional change in the concentration of a reactant critical to the germination process. From this assumption, the relationships of chemical kinetics could be used to describe the response of germination rate to stratification period (Chang 1977).

The velocity of an nth order reaction is proportional to the nth power of the concentration of the reactant, thus

$$v = k[A]^n,$$

where,

v = velocity,
k is a constant,
[A] is the concentration of the reactant, and
n is the proportionality coefficient.

The value of n may be obtained by measuring v at several concentrations of A and plotting log v versus log [A]. Generally, initial velocities (V_o) are used because the accumulation of reaction products can affect subsequent velocities. In the case of germination, mean rates were used rather than initial rates because individual seeds within a family constituted a population and their rates were normally, or very close to normally, distributed when time to germination was expressed as t^{-1} (Campbell and Sorensen, in prep.).

If reaction velocity is equated to germination rate and concentration of the reactant equated to length of stratification, the above equation becomes,

$$R = k[S]^n,$$

where,

R = mean germination rate (days^{-1} to 50 percent total germination),
k = constant,
[S] = duration of stratification (days), and
n = slope of the line relating R and [S].

Taking the common logarithms gives,

$$\log R = \log k + n \log [S]. \quad \text{(Eq. 1)}$$

When regression lines were compared statistically, the method of Snedecor and Cochran (1967, p. 432) was used.

Seedling Characters

Preliminary tests had indicated the total stratification + germination time needed to have germination of each treatment peak the last week in April, the time set for sowing. In actuality, three replications were sown April 26, one April 28, and one May 2. The delay was due to slow germination in seedlots with less stratification. This had two consequences. First, germinated seeds from faster germinating treatments (e.g., 64- and 128-day stratification) had to be returned to the cooler after germination to prevent further radicle development. Second, the slower germinating seeds in the short stratification treatments were not included in the seedling test. That is, the germinated seeds from the short-stratification treatments represented the portion of the population which had the greatest response to stratification. This was not true of the long-stratification treatments, because their seeds had essentially completed germination by the sowing dates.

The seedling test was established using germinated seeds from each of the 16 treatments in the germination test. Seeds were sown in eight-seedling rowplots.

The following traits were measured and analyzed.

1. Hypocotyl length.
2. Elongation rate between June 15 and August 24. Total height was measured every 2 weeks during the 1st year starting June 15. Plots of log height against time were linear between June 15 and August 24. The slope of this line (elongation rate) was determined for each rowplot.
3. Date of final budset, 1st year. Observations of the terminal growing point were made weekly, and a bud was considered set when green scales could first be seen at the base of the needles.
4. Total 1st-year height.
5. Total height after 2 years.
6. Diameter below the cotyledons after 2 years.

Seedling data was analyzed as a 2x8 factorial in a randomized complete block design with five replications. Rowplot means were used as the unit of observation. All treatments were fixed. Expected mean squares are:

Sources of variation	d.f.		Expected mean squares
Total	79		
Replication (R)	4		
Collection date (C)	1		$\sigma^2 + rs\ \sigma_C^2$
Stratification period (S)	7		$\sigma^2 + cr\ \sigma_S^2$
Linear		1	
Nonlinear		6	
C x S	7		$\sigma^2 + r\ \sigma^2_{CS}$
C x S linear		1	
C x S nonlinear		6	
Remainder	60		σ^2

RESULTS

Cone and Seed Measurements

Fresh and dry weights and moisture percentages of cones from the early collection were somewhat greater than for late-collected material (23.9 versus 21.2 g, 10.4 versus 10.1 g, and 128 versus 110-percent H_2O, respectively). Comparing moisture percentages with those reported in Ching and Ching (1962) indicates that the cones in the late collection were still about 2 weeks from full maturity. The time of natural seedfall was not observed, but it was assumed that the early collection was about 6 weeks and the late collection about 2 weeks prior to seedfall.

Filled seed weights from early and late collections were 10.2 and 12.6 mg, respectively.

Germination

Both stratification period and seed maturity affected percent germination of full firm seeds after 4 months in the germinator (fig. 1). For late-collected seeds, the relationship was linear with log [S]--each doubling of the stratification period increased total germination by an average of about 3 percent. Full germination (98 percent for this material), however, appeared to be achieved with about 30 days of stratification. For early-collected seeds, total germination was lower and the relationship to stratification period was curvilinear. Germination percentage increased for up to 16 days of stratification; stratification periods longer than 32 days appeared to be detrimental.

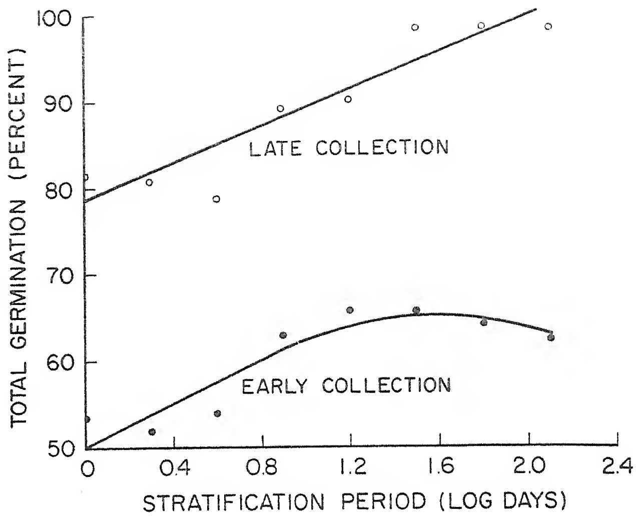

Figure 1.--Effect of stratification period on total germination percent of full, firm Douglas-fir seeds collected approximately 6 and 2 weeks before natural seed fall. ● = early collection; o = late collection.

The effects of seed maturity and length of stratification period on germination rate are shown in fig. 2. With short stratification, the early-collected seeds germinated more rapidly than the late-collected seeds. As length of stratification was increased, the difference in rates due to collection date decreased.

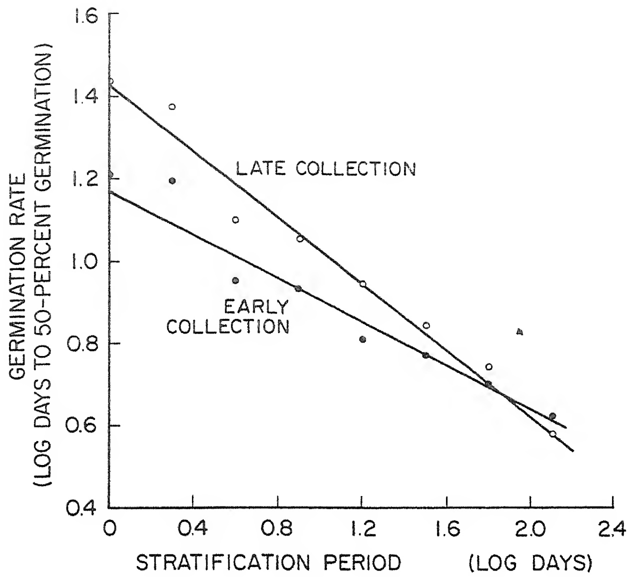

Figure 2.--Regression of germination rates of Douglas-fir seeds collected approximately 6 and 2 weeks prior to natural seed fall on stratification period. ● = early collection; o = late collection. The slopes of the lines are significantly different ($F=19.31$; d.f.=1, 12; $p<0.01$).

Seedling Traits

The effect of cone collection date on seedling size was significant for early measurements but did decrease with age (table 1). At the end of 2 years in the nursery, significant height differences had disappeared but significant diameter differences were still present.

Length of stratification affected size of seedlings grown from late-collected seeds but had little or no effect on seedlings grown from early-collected seeds. The relationship to 1st-year total height is shown in fig. 3. This relationship between stratification period and height was no longer significant for either seed maturity by the end of the 2d year in the nursery.

Table 1--Effect of date of cone collection on Douglas-fir seed and seedling traits. Early collection was made approximately 6 weeks and late collection approximately 2 weeks before natural seed fall

Trait (unit)	Cone collection date		Percent of decrease	Significance
	Early	Late		
Filled-seed weight (mg)	10.2	12.6	19	[1]0.01
Hypocotyl length (cm)	1.41	1.51	7	.01
Elongation rate[2]	.176	.176	0	n.s.
1-year total height (cm)	15.5	16.8	8	.01
1-year bud set (date)	Oct. 3	Oct. 3	0	n.s.
2-year total height (cm)	34.3	35.5	3	n.s.
2-year diameter (mm)	6.07	6.45	6	.01

[1]Significance of 0.01 indicates that the probability of no difference due to date of cone collection 0.01, n.s. = nonsignificant.

[2]Cm/cm per 2 weeks between June 15 and August 24 of the 1st year in nursery.

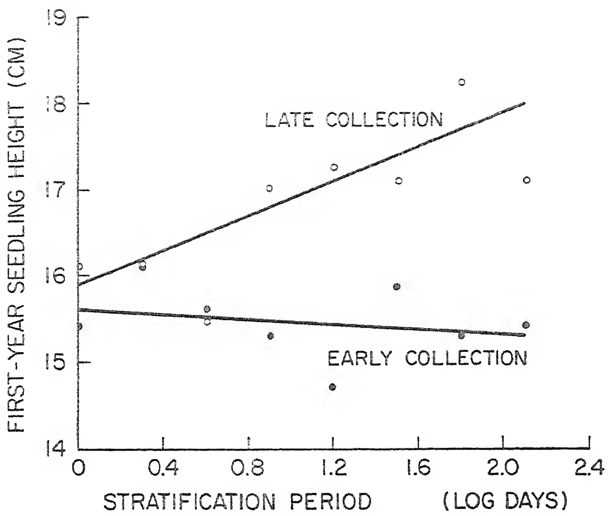

Figure 3.--Effect of seed collection date and stratification period on 1-year heights of Douglas-fir seedlings. ● = early collection; o = late collection. Each point is the mean of five replications. The slopes of the two lines are significantly different ($F=5.91$; d.f.=1, 60; $p < 0.05$).

DISCUSSION

Germination

Total germination of filled late-collected seeds was 78 percent or better following all lengths of stratification; nevertheless germination improved significantly when seeds were stratified for periods up to 1 month. Thirty days of cold, moist storage was sufficient to raise total germination of mature seeds to 98 percent.

Total germination of filled early-collected seeds was lower. Also, early-collected seeds responded to longer stratification periods differently than late-collected seeds. Although Allen (1958) reported that stratification appeared to have a detrimental effect on most of the "immature" seed in "immature" lots, Ching and Ching (1962) found stratification to be beneficial to seeds of all maturities. They suggested that differences in stratification procedures might explain why their results differed from those of Allen. My results indicate one aspect of stratification procedure, length of stratification, does influence total germination percentage of immature seeds--short stratification periods being beneficial and periods beyond about 30 days detrimental.

The log of days to 50 percent germination was closely correlated with log of stratification period (fig. 2). Examination of a limited amount of other data (Allen 1962, Sorensen, unpubl.) indicated that the relationship may be general but that the slope of the line may vary for seeds of different geographic origins as well as for seeds of different maturities. The lines in fig. 2 indicate that four to six stratification periods equally spaced on the log time scale, starting with a short stratification period and extending to about 10 weeks stratification should characterize the relationship between stratification period and germination rate for Douglas-fir seeds.

Germination rates of early-collected seeds were not affected by stratification as much as were the germination rates of late-collected seeds. If germination rates after little or no stratification indicate degree of embryo dormancy or rest, then the early-collected seeds in this study were less dormant initially than the late-collected seeds. Since dormancy may deepen in cold storage for loblolly pine (Pinus taeda L.) (McLemore and Barnett 1966), storage time may also affect the response to stratification period.

Seedling Growth

In this test, weights of early-collected seeds were 19 percent less than weights of late-collected seeds and seedlings grown from early-collected seeds averaged 8 percent shorter than seedlings grown from late-collected seeds (table 1). In previous studies in which Douglas-fir seedlings were raised from seeds of different weights (Sorensen 1973 and unpubl.), it was estimated that a 10-percent difference in seed weight was accompanied by a difference in 1st-year height of about 3-5 percent. Thus, there was no evidence that the reduction in seedling vigor associated with early cone collection was due to factors other than seed size.

The results indicated that the direct effect of stratification on late-collected seeds continued during epicotyl extension but not thereafter. Although the relation between stratification period and 1st-year height was significant, this was apparently the result of the relationship between stratification period and hypocotyl length, because stratification period did not influence either elongation rate during the exponential phase of stem extension or length of the growing season.

It is probable that stratification period would usually have a greater indirect effect on seedling size through its effect on onset of germination than it does through its direct effect on hypocotyl extension. In this test, pregerminated seeds were sown. Consequently, all seedlings started nursery growth at the same time. The germination rate results show, however, that if ungerminated seeds with different stratification periods had been sown in the nursery, they would have germinated and started growth at greatly different times. Further, our germination test was conducted at 20°C. Nursery soils are often cooler than this at the time of sowing. Allen (1960) reported that decreasing the length of stratification decreased the rate of low-temperature germination (10°C) of Douglas-fir seeds even more than it did the rates of germination at higher temperatures. Because the date at which seedling growth starts can influence both the length of the growing season and the relationship of particular developmental phases with the annual climatic cycle, the date of germination could have a large effect on seedling size and form (Sorensen 1978).

McLemore also concluded that the differences in size of seedlings from stratified and unstratified loblolly pine seed was due to earlier germination of the former.[1/] Lavender[2/] has made similar observations in a Douglas-fir nursery. Lavender also noted that there appeared to be an interaction between the effects of stratification period on germination rate and date of sowing in the nursery. This was apparently due to the change in nursery soil temperature during the spring (Lavender, unpubl.) and is in line with the experimental results of Allen (1960) and with other observations on the interacting effects of stratification period and germination temperature on germination rate (Vegis 1963).

LITERATURE CITED

Allen, G. S.
1958. Factors affecting the viability and germination behavior of coniferous seed. II. Cone and seed maturity, Pseudotsuga menziesii (Mirb.) Franco. For. Chron. 34:275-282.

Allen, G. S.
1960. Factors affecting the viability and germination behavior of coniferous seed. IV. Stratification period and incubation temperature, Pseudotsuga menziesii (Mirb.) Franco. For. Chron. 36:18-29.

[1/]Personal communication, 1979. B. F. McLemore, USDA, Southern Forest Experiment Station, Monticello, Arkansas.

[2/]Personal communication, 1979. D. P. Lavender, Department of Forest Management, Oregon State University, Corvallis.

Allen, G. S.
1962. Factors affecting the viability and germination behavior of coniferous seed. VI. Stratification and subsequent treatment, Pseudotsuga menziesii (Mirb.) Franco. For. Chron. 38:485-496.

Campbell, R. K., and A. I. Sugano.
1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. Bot. Gaz. 140(2):223-231.

Chang, R.
1977. Physical chemistry with applications to biological systems. MacMillan Publ. Co., Inc. New York, 538 p.

Ching, T. M., and K. K. Ching.
1962. Physical and physiological changes in maturing Douglas-fir cones and seeds. For. Sci. 8:21-31.

Cram, W. H., and H. A. Worden.
1957. Maturity of white spruce cones and seed. For. Sci. 3:263-269.

Flemion, F.
1934. Dwarf seedlings from non-after-ripened embryos of peach, apple, and hawthorn. Contrib. from Boyce Thompson Inst. 6:205-209.

Kriek, W.
1976. Early results of the Sitka spruce ten provenance experiment at Wageningen, the Netherlands. In J. O'Driscoll (ed.), IUFRO Sitka spruce international ten provenance experiment. Nursery stage results. Dep. of Lands, For. and Wildl. Serv., Dublin, Ireland, p. 173-205.

McLemore, B. F., and J. P. Barnett.
1966. Loblolly seed dormancy influenced by cone and seed handling procedures and parent tree. USDA, For. Serv. Res. Note SO-41, 4 p. South. For. Exp. Stn., New Orleans, La.

McLemore, B. F., and F. J. Czabator.
1961. Length of stratification and germination of loblolly pine seed. J. For. 59:267-269.

Maki, T. T.
1940. Significance and applicability of seed maturity indices for ponderosa pine. J. For. 38:55-60.

Mergen, F.
1963. Ecotypic variation in Pinus strobus L. Ecology 44:716-727.

Nienstaedt, H.
1967. Chilling requirements in seven Picea species. Silvae Genet. 16:65-68.

Olson, D. L., and R. R. Silen.
1975. Influence of date of cone collection on Douglas-fir seed processing and germination: a case history. USDA For. Serv. Res. Pap. PNW-190. 10 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oreg.

Pollock, B.
1959. Temperature control of physiological dwarfing in peach seedlings. Nature 183:1687-1688.

Rediske, J. H.
1969. Effects of cone-picking date on Douglas-fir seed quality. For. Sci. 15:404-410.

Rohmeder, E.
1962. Die Bedeutung der Keimschnelligkeit bei forstlichen Samenarten. Int. Seed Test. Assoc. Proc. 27:657-671.

Schwabe, W. W.
1973. Morphogenetic effects of light and temperature in relation to crop yield. Angewandte Botanik 47:1-16.

Snedecor, G. W., and W. G. Cochran.
1967. Statistical Methods. 6th Ed. Iowa State Univ. Press, Ames. 593 p.

Sorensen, F. C.
1973. Performance of wind-pollinated families and intra- and inter-stand crosses on contrasting forest soils. USDA For. Serv. Res. Note PNW-207, 7 p. Pac. Northwest For. and Range Exp. Stn., Portland, Oreg.

Sorensen, F. C.
1978. Date of sowing and nursery growth of provenance of Pseudotsuga menziesii given two fertilizer regimes. J. Appl. Ecol. 15:273-279.

Vegis, A.
1963. ,Climatic control of germination, bud break, and dormancy. In Environmental Control of Plant Growth. L. T. Evans, ed. Acad. Press, New York, p. 265-287.

Wommack, D. E.
1964. Temperature effects on the growth of Douglas-fir. Ph.D. thesis. Oreg. State Univ., Corvallis.

GPO 990-095

185

The FOREST SERVICE of the U.S. Department of Agriculture is dedicated to the principle of multiple use management of the Nation's forest resources for sustained yields of wood, water, forage, wildlife, and recreation. Through forestry research, cooperation with the States and private forest owners, and management of the National Forests and National Grasslands, it strives — as directed by Congress — to provide increasingly greater service to a growing Nation.

The U.S. Department of Agriculture is an Equal Opportunity Employer. Applicants for all Department programs will be given equal consideration without regard to age, race, color, sex, religion, or national origin.